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Low Dose Interleukin-2 for Refractory Autoimmune Hepatitis

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Abbreviations:

AIH – Autoimmune Hepatitis

ANA – Anti-neutrophil Antibody

AST – Aspartate Aminotransferase

CXCL10 – C-X-C Motif Chemokine Ligand 10

FOXP3 – Forkhead-box-P3

IgG – Immunoglobulin G

IL-2 – Interleukin-2

IL-10 – Interleukin-10

IP-10 – Interferon-Inducible Protein-10

IQR – Interquartile Range

LDIL-2 – Low Dose Interleukin-2

MFI – Mean Fluorescence Intensity

MMF – Mycophenolate Mofetil

pSTAT5 – Phosphorylated Signal Transducer and Activator of Transcription-5

SEM – Standard Error of Mean

SLA – Anti-Soluble Liver Antigen

SMA – Anti-Smooth Muscle Antibody

Tcon – $CD4^{+}CD25^{-}FoxP3^{-}$ T Conventional Cell

Treg – $CD4^{+}CD25^{+}Fopx3^{+}$ T Regulatory Cell

Keywords: Autoimmune hepatitis; interleukin-2; regulatory T-cells

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INTRODUCTION

The management of autoimmune hepatitis (AIH) has largely remained unchanged for the past three decades. The optimal regimen for patients who do not respond to first-line treatment of corticosteroids with-or-without azathioprine remains unclear. There is growing interest in the use of immunotherapies targeting $CD4^{+}$Forkhead-box-P3$(Foxp3)^{+}$ regulatory T-cells (Tregs). Tregs are a T-lymphocyte sub-population with immunosuppressive and cytoprotective capacity that play a key role in restraining auto-reactive conventional T-lymphocytes (Tcons) and preventing autoimmunity. Due to their constitutive expression of CD25, the alpha-chain of the interleukin-2-receptor, Tregs are highly dependent on, and exquisitely sensitive to, IL-2.(1) The use of low-dose IL-2 (LDIL-2) as an immunomodulatory agent is being explored in various autoimmune disorders.(2) We report here the clinical and immunological effects of LDIL-2 in two patients with refractory AIH.

Patients

Patient-1 had previously required repeated intravenous corticosteroid courses for AIH flares despite triple therapy. Patient-2 was intolerant to azathioprine, had suffered multiple flares whilst on MMF and prednisolone, and refused initiating Tacrolimus due to long-term toxicity concerns. Both patients had persistent disease activity as assessed by serum biochemistry and a liver biopsy performed within the previous 12months (Table_1). IL-2 (Proleukin, Novartis Pharmaceuticals UK) was administered as 1million IU subcutaneous injections for 5 consecutive days monthly, for 6months. Patients gave written informed consent to receive LDIL-2 under a clinical protocol approved by King's Medicines

Management Committee, and to participate in an immunomonitoring study (REC15/NS/0062) involving collection of sequential peripheral blood samples.

Patient-1 experienced no significant changes in aspartate aminotransferase (AST) and immunoglobulin-G (IgG) levels between baseline and end-of-treatment. Conversely, in patient-2 both AST and IgG levels decreased to within the normal range by end-of-treatment (Table_1 and Fig.1A). No adverse events other than rapidly resolving mild injection-site reactions were observed.

The proportion of circulating Tregs increased significantly in both patients by day-4 after each cycle of LDIL-2, peaking at day-9, and returned to baseline values at day-28 (Fig.1B). The changes in Tregs were due to increases in the proportion of resting ($CD45RA^{+}FOXP3^{lo}$) and effector Tregs ($CD45RA^{-}FOXP3^{hi}$), with no significant changes in the cytokine-secreting, non-suppressive $CD45RA^{-}FOXP3^{lo}$ subset (Sup_Fig.1A-B). LDIL-2 therapy also resulted in increases in soluble CD25, Treg proliferation (as assessed by Ki-67), and in the Treg expression of CD25 and FOXP3 (considered surrogate markers of suppressive capacity) (Sup_Figs.1C-E, 3B & 5F-G). We measured intra-cellular pSTAT5 to assess the IL-2 Treg sensitivity. At baseline, Tregs from both patients exhibited lower pSTAT5 levels than those from age-matched healthy controls, but this was normalized following LDIL-2 therapy (Fig.1D).

DISCUSSION

We provide here what to the best of our knowledge is the first evidence suggesting that administration of LDIL-2 to AIH patients is safe and effective in increasing the pool of circulating Tregs. While we observed a consistent increase in Tregs following LDIL-2 therapy, this effect was of shorter duration and lower

magnitude than that reported in patients receiving higher and/or more frequent doses (1-3millionIU/day),(2) suggesting these alternative regimens might be preferable to sustain the expanded Treg pool. Of note, LDIL-2 treatment increased the IL-2 sensitivity of Tregs and corrected the suboptimal pSTAT5 response recently described as being a hallmark of AIH.(3) Importantly, the effects of LDIL-2 were restricted to Tregs, as we detected no significant changes in non-Treg immune cell subsets (Fig.1C and Supplementary Results). Animal data indicate that intra-hepatic Treg expansion is compromised in the presence of severe liver damage.(4) This could account for patient-1 lack of clinical response.

In conclusion, our observations demonstrate the feasibility of using LDIL-2 to improve inflammatory liver damage by increasing the pool of immunoregulatory lymphocytes. Our data provide rationale and a proof-of-principle to further explore the clinical use of LDIL-2 in the management of autoimmune liver diseases.

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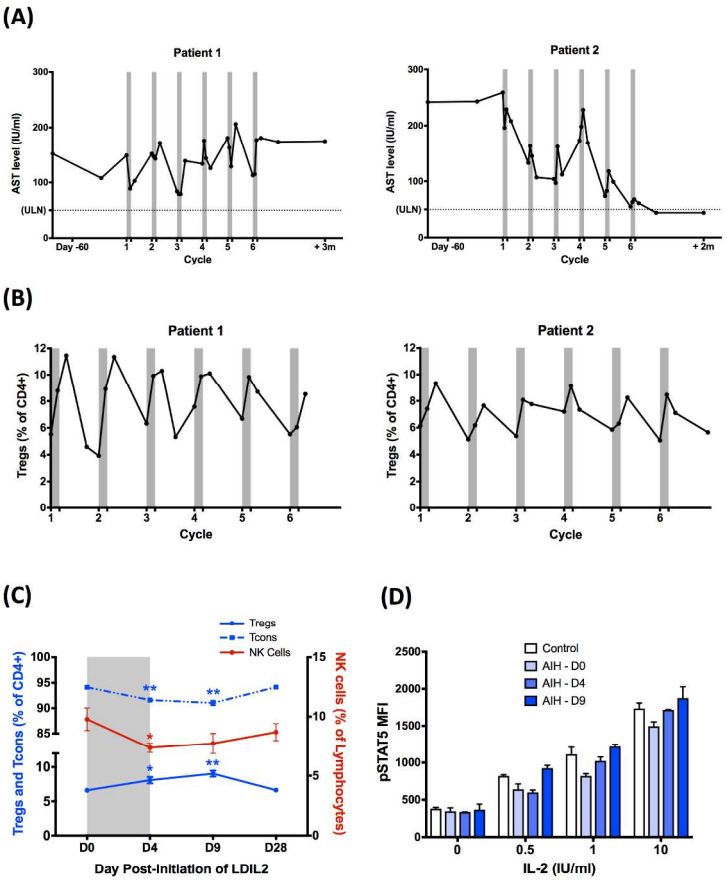
Table 1. Clinical characteristics of the 2 patients receiving low-dose interleukin-2 (LDIL-2) treatment. Baseline serum tests were taken prior to initiating LDIL-2 treatment, whilst end of treatment was taken at 3 months following completion of last (sixth) cycle of LDIL-2 treatment.

Patient Characteristics	**Patient-1**	**Patient-2**
Age (years)	20	56
Sex	Female	Female
Age at diagnosis (years)	13	51
Duration of disease (years)	7	5
Serology	SMA+ (1/160)	ANA+ (1/40) Anti-SLA +
IAIHG Score	8	9
Immunosuppression at baseline	Tacrolimus 1mg OD MMF 1g BD Prednisolone 15mg OD	MMF 1g BD Prednisolone 15mg OD
Liver histology	Severe autoimmune hepatitis with cirrhosis	Moderate chronic hepatitis with interface activity and focal bridging fibrosis
Baseline serum tests (normal range)		
• AST *(10-50 IU/ml)*	149	259
• Globulin *(25-35 g/l)*	53	43
• Immunoglobulin G *(6.3-18.1 g/l)*	29.4	20.9
• Albumin *(35-50 g/l)*	42	44
• Bilirubin *(3-20 µmol/l)*	40	14
• Platelet *(150-450 x 10^9/l)*	152	198
• INR *(0.90-1.20)*	1.17	1.11
Serum tests at end-of-treatment		
• AST *(10-50 IU/ml)*	174	44
• Globulin *(25-35 g/l)*	49	33
• Immunoglobulin G *(6.3-18.1 g/l)*	30.2	16.2
• Albumin *(35-50 g/l)*	37	41
• Bilirubin *(3-20 µmol/l)*	33	55
• Platelet *(150-450 x 10^9/l)*	132	191
• INR *(0.90-1.20)*	1.07	1.06

*SMA – smooth muscle antibodies, ANA – anti-neutrophil antibody, Anti-SLA – anti-soluble liver antigen, MMF – Mycophenolate mofetil, AST – aspartate aminotransferase, OD – Once Daily, BD – bis die/twice daily, INR – International Normalised Ratio.

Fig. 1. Effect of low-dose interleukin-2 (LDIL-2) 5-day treatment cycles, (depicted in grey shade) on serum biochemistry and circulating $CD4^+CD25^+FoxP3^+$ regulatory T-cells (Tregs). (A) Sequential changes in serum AST levels during the 6 cycles of LDIL-2 treatment for each patient. **(B)** Proportion of Tregs before and after each of the cycles of LDIL-2 treatment.. **(C)** Changes in the proportion of Tregs, conventional T-cells (Tcons) and natural killer (NK) cells after combining the data from the 12 cycles of LDIL-2 treatment from both patients (data show mean and standard error of mean, SEM, * = p-value <0.05, ** = p-value <0.005). **(D)** Sensitivity to IL-2 of circulating Tregs collected before and at different time points after initiating LDIL-2. The response to IL-2 was assessed by measuring in Tregs the mean fluorescence intensity of intracellular pSTAT5 (pSTAT5 MFI; y-axis) by flow cytomery following *ex vivo* stimulation with increasing concentrations of IL-2 (x-axis). Data shown correspond to 3 healthy controls and 2 patients and are expressed as mean ± SEM.



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